

Remarks/Arguments:

I. Status of the Application

Filed concurrently with this paper is a Request for Continued Examination (RCE) under 37 C.F.R. §1.114. Accordingly, the finality of the Office Action mailed on August 7, 2009 should be withdrawn and this reply is timely and proper. Applicants respectfully request consideration of the claim amendments and remarks set forth in this paper.

II. Remarks Concerning the Technical Field of the Invention

For a better understanding of the present invention, Applicants believe it would be appropriate to summarize the uniqueness of the cell walls of mycobacteria and the particularities of tuberculosis disease, tuberculosis infection and its prophylaxis and treatment.

II.1. Uniqueness of the cell wall of mycobacteria

The envelope of mycobacteria is composed of the plasma membrane and a cell wall surrounding such membrane. The cell wall of *M. tuberculosis* is thick and waxy and it is a major determinant of virulence for the bacterium.

The cell wall of *M. tuberculosis* is highly complex in comparison with the cell wall of other Gram positive bacteria. It is characterized by its high content in lipids and complex polysaccharides.

The cell wall is mainly defined as a skeleton formed by:

- a covalently linked layer of peptidoglycan;
- arabinogalactan, i.e., a branched-chain polysaccharide of arabinose and galactose; and
- mycolic acids, which are high-molecular weight fatty acids of sizes and structures unique to mycobacteria;

wherein the arabinogalactan is covalently linked to the peptidoglycan layer by phosphodiester bonds and the arabinogalactan distal ends are esterified with the mycolic acids (see Figure 1, below). This cell wall skeleton is designated as "mycolyl-arabinogalactan-peptidoglycan complex (mAGP)".

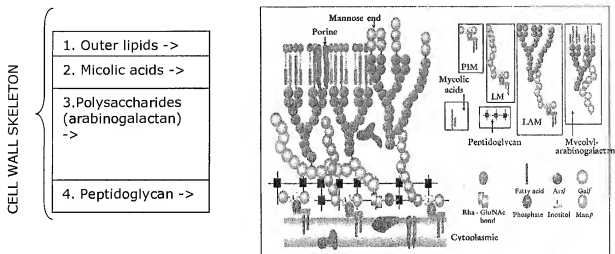
Outer from the mycolic acids, there are free lipids and glycolipids that are not covalently bound to the wall.

Together with these lipids, there are proteins and polysaccharides constituted by arabinose and mannose (arabinomannan or lipoarabinomannan (LAM)) and glucose (glucans). These polysaccharides are called complex glycans (GLI).

It also contains some proteins.

Some of the compounds of the mycobacterial cell wall are toxic compounds, such as trehalose 6,6'-dimycolate (also known as cord factor, most abundantly produced by virulent mycobacterial strains).

Figure 1. Illustration of the cell wall of *Mycobacterium tuberculosis*:



II.2. Particularities of tuberculosis disease, tuberculosis infection and its prophylaxis and treatment

The distinction between tuberculosis infection (also known as Latent Tuberculosis Infection (LTBI)) and tuberculosis disease should be explained.

A person becomes infected with tuberculosis when a strain of *Mycobacterium tuberculosis* complex (MTB-C) reaches the alveoli in the lungs.

Infected people do not present symptoms and cannot spread the disease, but give a positive to the tuberculin test. A person may remain infected for years. It is believed that there are 2,000,000,000 people infected with tuberculosis worldwide.

Several hypotheses for explaining LTBI have been proposed. LTBI is mainly characterized by "latent" or non-replicating bacilli. It has been suggested that "latent" bacilli survive in granulomas with a central necrotic core and surrounded by an outermost layer of foamy macrophages. This outermost layer is believed to constitute an immunosuppressive barrier.

Some factors may activate the latent bacilli and the patient may then develop the disease presenting typical symptoms due to actively growing bacilli. It is estimated that over 10 % of infected people will develop the disease.

Latent bacilli have been observed to be able to survive in more stressful conditions than actively growing bacilli. Currently, not much information is available regarding antigens characteristic of latent bacilli.

Current standard treatment to fight tuberculosis in people having LTBI or already having developed the disease consists of the administration of several drugs (chemotherapy), including isoniazid, for a long period.

Chemotherapy acts on metabolically active growing bacilli. After a short period of chemotherapy most of the metabolically active growing bacilli are supposed to be eliminated. However, latent bacilli still persist, leading to a state of chronic tuberculosis infection. In order to completely eliminate the infection a long-term period of from 6-9 months of chemotherapy is needed.

This long-term therapy makes treatment-compliance extremely difficult. If the treatment is stopped before its end, the patient may develop the disease again. Further, non-compliance may induce the development of strains resistant to chemotherapy, which make the eradication of tuberculosis more difficult.

It is important to distinguish between the profilaxis and the therapeutic treatment of tuberculosis.

As prophylactic measures, an immunogenic vaccine is given to uninfected patients. Current vaccine is based on bacteria of the so-called bacillus Calmette-Guerin (BCG) strain, an attenuated variety of *M. bovis* developed more than 60 years ago. In fact, BCG vaccination can prevent the disease though not the infection. This prophylactic treatment with current BCG vaccine should not be given to infected people, since severe toxic reactions have been observed.

Other vaccines probed to be immunogenic when given to uninfected mammals have also shown to be ineffective or even cause severe toxic reactions when given to infected mammals, resulting in ineffective treatment of tuberculosis and raising safety concerns.

There is a great need to develop a safe treatment for tuberculosis and LTBI which is capable of reducing the need for long-term chemotherapy treatment.

III. Status of the Claims

In order solely to expedite prosecution and in no way conceding to the rejection, Applicants have cancelled claims 20, 28-34 and 38-43, without prejudice.

Claim 47 has been amended by including steps c) and d) of claim 53. Dependent claim 53 has been amended accordingly by deleting steps c) and d). Support for these amendments may be found in page 4 of the specification. No new matter has been added.

Claims 13-19, 21-27, 35-37, 45 and 46 are withdrawn. As a result, claims 47-60 are currently pending and under examination.

IV. Claim Objections

Claims 20, 28 and 29 were objected to for requiring the particulars of withdrawn claims. Since claims 20, 28 and 29 have now been cancelled, the objection is rendered moot.

V. Rejections under 35 USC §103

The Examiner has reiterated the obviousness rejections set forth in the previous Office Action mailed March 11, 2009. Since claims 20, 28-34 and 38-43 have been cancelled without prejudice, the rejections against these claims are rendered moot.

V.1 Rejection over Andersen *et al.* in view of Chaturvedi *et al.*

Claims 47-55 have been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Andersen *et al.* (U.S. 2002/0094336) in view of Chaturvedi *et al.* (Vaccine 17:2882-2887; 1999).

The Examiner reiterates the arguments made in the previous Office Action. The Applicants respectfully traverse.

To establish a *prima facie* case of obviousness:

- the cited references must disclose all of the claim limitations; and
- there must be a reasonable expectation of success.

The Applicants respectfully submit that these requirements are not fulfilled in the present case and that therefore the obviousness rejection should be withdrawn.

Claim 47 is a product-by-process claim.

The immunotherapeutic agent of the instant invention is characterized by being obtained by a process which includes the steps of:

- a) culturing the cells of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain for a period of at least three weeks; and
- b) homogenizing the cells in the presence of a non-ionic surfactant selected from the group consisting of alkylphenol ethoxylates and ethoxylated sorbitan esters, to produce a homogenate comprising non-fragmented cells, cell wall fragments, and solubilized cell compounds.

The cited prior art references, in particular Andersen *et al.* and Chaturvedi *et al.*, do not disclose these steps. These steps of the process (i.e. the culture period, and the fact that the homogenization is carried out in the presence of a non-ionic surfactant) will impart distinctive structural characteristics to the final product.

Further, a person having ordinary skill in the art would not have had a reasonable expectation that the instantly claimed immunotherapeutic agent would have the observed activity.

This is supported by Dr. Cardona's declaration, which accompanies this Amendment and which is further discussed below.

i) The amended claims better clarify that the immunotherapeutic agent corresponds to the non-solubilized cell wall fraction

The Examiner has argued that the claimed immunotherapeutic agent obtained by a process only defined by comprising steps a) and b) would be contrary to Applicants' own specification and may encompass virulent non-fragmented cells and likely lead to tuberculosis in the recipient subject.

In order solely to expedite prosecution and in no way conceding to the rejection, Applicants have amended claim 47. Currently amended claim 47 now recites an immunotherapeutic agent obtained by a process also comprising a step involving the separation of the cell wall fragments by centrifugation so as to remove non-fragmented cells and to discard solubilized cell compounds in the supernatant, followed by washing of the cell wall fragments and inactivation of any remaining virulent cells. Thus, amended claim 47 makes clearer that the immunotherapeutic agent corresponds to the non-solubilized cell wall fraction, in agreement with Applicants' own specification. Therefore, it is respectfully submitted that currently amended claim 47 renders moot the above-mentioned Examiner's argument.

Claim 47 does not include step e) of claim 53 regarding the lyophilization of the immunotherapeutic agent. This would be in agreement with Dr. Cardona's declaration submitted herewith, according to which the immunotherapeutic agent instantly claimed would have the same biological activity before lyophilization.

ii) Effect of culture time

Neither Andersen *et al.* or Chaturvedi *et al.* disclose a process for preparing an immunotherapeutic agent comprising cell wall fragments of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain of cells comprising culturing such cells for a period of at least three weeks.

With regard to the culturing of the mycobacterium for at least three weeks, such has not been accorded patentable weight by the Examiner. According to the Examiner, "culturing time has been considered to be within the purview of a person of ordinary skill in the art and constitutes routine optimization to obtain a desired yield of mycobacteria, that include scale-up culture of slow growing bacteria."

The culturing time recited in Applicants' claims is relatively long (e.g., as compared to the few days of short-term culture filtrate (ST-CF) disclosed in Andersen *et al.* cf. paragraphs [0006], [0009], example 3) or the culturing time of 10 days employed in the process disclosed in Chaturvedi *et al.*

The culture time has not been chosen to obtain the highest yield of mycobacteria. In fact, such a long time would not be optimal for achieving maximal cell density and viability, as supported by Dr. Cardona's declaration. Thus, the skilled person would not have a reason to chose such a long culturing time.

Dr. Cardona's declaration explains the effect of this feature (i.e., a different antigenic profile is observed when the culturing time is increased to at least three weeks) as well as the reason for such an effect (i.e., the induction of (stressful) conditions similar to those found where the

tuberculosis latent bacilli may survive, thus favoring the production of antigens typical from latent bacilli).

Further, as supported by Dr. Cardona's declaration, a shorter culturing time such as that disclosed in Andersen *et al.* or Chaturvedi *et al.* would not have induced the same conditions. Thus, such shorter culturing time would not result in an agent with activity comparable to the immunotherapeutic agent instantly claimed.

Therefore, this feature is not the result of routine optimization. Rather, it results in structural differences and is relevant to the activity of the immunotherapeutic agent instantly claimed.

It is respectfully submitted that the teachings of Andersen *et al.* and Chaturvedi *et al.*, taken alone or in combination, would not have led a person of ordinary skill in the art to reasonably expect that an immunotherapeutic agent prepared by isolating cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain using a process comprising a step of culturing the cells for at least three weeks, as instantly claimed, would have improved or enhanced activity. Combining and modifying the prior art disclosures so as to arrive at Applicants' claimed invention thus would not have been obvious.

iii) *Effect of homogenizing cells in the presence of the claimed non-ionic surfactant*

As explained in Section II herein, the cell wall of mycobacteria, more in particular of MTB-C strain cells, is characterized by its high content in lipids and complex polysaccharides. It also contains some proteins.

Non-ionic surfactants selected from the group consisting of alkylphenol ethoxylates and ethoxylated sorbitan esters, such as Triton® X-114, are known to be able to break lipid-lipid interactions and protein-lipid interactions and so to solubilize lipids such as those of biological membranes and to solubilize some proteins.

The effect of homogenizing cells in the presence of the claimed non-ionic surfactant is explained in Dr. Cardona's declaration. During the homogenization, the non-ionic surfactant solubilizes at least part of the lipid compounds and proteins of the cell wall of the *M. tuberculosis*. Therefore, the immunotherapeutic agent corresponding to the non-solubilized cell wall fraction as instantly claimed:

- will lack the cell wall lipids and cell wall proteins solubilized by the surfactant treatment, some of which may be toxic;
- will contain non-solubilized proteins which would be devoid from most of the surrounding lipids and therefore more exposed; and
- will contain non-solubilized lipids, which will be finely divided by the emulsion formed during the homogenization with the surfactant.

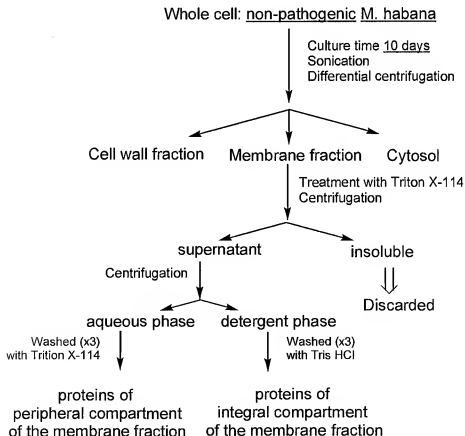
Thus, as supported by Dr. Cardona's declaration, the fact that the homogenization step is performed in the presence of the instantly claimed non-ionic surfactant, will impart distinctive structural characteristics to the final product, resulting in different biological activity.

iv) Neither Andersen *et al.* nor Chaturvedi *et al.* alone disclose a process for preparing an immunotherapeutic agent comprising cell wall fragments obtained by a process comprising a step of homogenizing the cells in the presence of the claimed non-ionic surfactant

The Examiner holds that Andersen *et al.* do not describe the preparation of Mycobacterial cell wall fragments. We remit to our previous response in this regard.

The Examiner considers that with regard to homogenization in the presence of a non-ionic surfactant, Chaturvedi *et al.* describe the preparation of protective antigens from the cell wall of *Mycobacterium habana* (Title and Abstract), by sonication, differential centrifugation and phase separation using Triton X-114, followed by further treatment of both the aqueous and detergent phases with Triton X-114. The Examiner concludes that there is no evidence that the cell wall fraction instantly claimed would be structurally or functionally distinct from that taught and suggested by the prior art.

Applicants respectfully disagree. Applicants do not understand the reason why the Examiner insists on considering the process for preparing cell wall fragments disclosed in Chaturvedi *et al.* as comprising a step of phase separation using Triton® X-114. The process for preparing the cell wall fraction in Chaturvedi *et al.* does not comprise a step of homogenizing the cells in the presence of a non-ionic surfactant. This was already clarified in Applicants' previous response. For further clarification, the process disclosed in Chaturvedi *et al.* has been schematized below:



The Examiner has noted that Applicants' method of homogenizing the cells in the presence of non-ionic surfactant necessarily includes treatment of the cell membrane fraction with Triton® X-114 as described by Chaturvedi *et al.* Further, the Examiner indicates that according to the specification all soluble fractions are separated from the cell wall fraction and discarded (that would include all proteins solubilized by the non-ionic surfactant), and only the insoluble cell wall fraction is used as an immunotherapeutic agent.

Applicants acknowledge that the homogenization of the cells in the presence of the claimed non-ionic surfactant may solubilize the proteins of the membrane. However, this merely amounts to indicating that the isolated cell wall fragments could be devoid of membrane proteins. It is respectfully submitted that the relevant point herein is the effect that the homogenization step will have on the composition and structure of the cell wall fragments obtained by the claimed process. This point is discussed in more detail below in the section regarding the effect of the homogenization step.

The Examiner has also argued that Applicants have ignored the teachings of Chaturvedi *et al.* with respect to using Triton® X-114 non-ionic surfactant in the isolation process.

Applicants respectfully submit that the teaching of Chaturvedi *et al.* regarding the use of Triton® X-114 non-ionic surfactant in the isolation process is limited to using the surfactant for treating the membrane fraction, in particular, for isolating the proteins from the peripheral compartment of the membrane from the proteins of the integral compartment of the membrane.

Thus, Chaturvedi *et al.* alone do not disclose, teach or suggest the preparation of cell wall fragments of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain by a process comprising a step of homogenizing the cells in the presence of the claimed non-ionic surfactant.

v) The combined teachings of Andersen et al. and Chaturvedi et al. do not disclose or suggest an immunotherapeutic agent comprising cell wall fragments obtained by a process comprising a step of homogenizing the cells in the presence of the claimed non-ionic surfactant

The combined disclosures of Andersen *et al.* and Chaturvedi *et al.* do not teach or suggest the preparation of cell wall fragments of a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain by a process comprising a step of homogenizing the cells in the presence of the claimed non-ionic surfactant.

The Examiner argues that Applicants have attacked the references individually and has not found persuasive the argument that the strain used in Chaturvedi *et al.* was the non-pathogenic *M. Habana* and not the virulent H37Rv strain since, e.g., Andersen *et al.* teach vaccine compositions of the H37Rv strain.

Applicants respectfully submit that the combined teachings of Andersen *et al.* and Chaturvedi *et al.* would not lead the person having ordinary skill in the art to apply the teachings of Chaturvedi *et al.* to the virulent strain H37Rv. Even if the teaching of Chaturvedi *et al.* was applied to the virulent H37Rv strain instead of being applied to the non-pathogenic *M. Habana*, that would not result in the immunotherapeutic agent instantly claimed. The effect of

performing the homogenization of the cells in the presence of the particular non-ionic surfactant(s) recited in Applicants' claims has been explained above.

As supported by Dr. Cardona's declaration, the process for preparing the cell wall fraction according to Chaturvedi *et al.*, (i.e., by sonication and centrifugation, lacking the step of homogenizing the cells in the presence of the selected non-ionic surfactant), even if applied to the virulent H37Rv strain, will result in a different composition than the immunotherapeutic agent instantly claimed and accordingly will have different properties.

vi) No reasonable expectation of success

To establish a valid *prima facie* case of obviousness, it is fundamental that there must be a reasonable expectation of success.

As discussed previously in Section II of this Amendment, it is important to distinguish between prophylaxis of tuberculosis, currently performed with immunogenic BCG vaccination, and treatment of tuberculosis currently performed with long-term chemotherapy treatments, both directed against growing bacilli.

Neither Andersen *et al.* nor Chaturvedi *et al.* disclose any example wherein the disclosed compositions are given to infected animals for the treatment of tuberculosis.

According to Dr. Cardona's declaration, several previous attempts by others to identify an immunotherapeutic agent useful for the treatment of tuberculosis failed. Even vaccines previously probed to be immunogenic when given to uninfected people were found to be ineffective when given as treatment to infected patients. Furthermore, the development of an immunotherapeutic agent would have raised safety concerns due to the recognition that known immunogenic compositions including the current prophylactic treatment with BCG vaccination induced severe toxic reactions when given to infected mammals.

Thus, as supported by Dr. Cardona's declaration, at the time the instant invention was made a person ordinarily skilled in the art would not have had a reasonable expectation of succeeding in finding an immunotherapeutic agent effective against latent tuberculosis, based on a non-purified cell extract of a virulent tuberculosis strain, that would result in substantially reducing the number of bacilli and, also importantly, without inducing toxic responses, so that it could be potentially used to significantly reduce the currently employed long-term (6 to 9 month) chemotherapy treatment.

Dr. Cardona's declaration also discusses examples of the application, in particular example 3 trial 4 effectively showing the unexpected activity of the immunotherapeutic agent instantly claimed which after a very short chemotherapy treatment significantly reduces the number of bacilli including latent bacilli in the lungs in a murine model of chronic tuberculosis infection. Despite being a non-purified mixture of antigens obtained from a virulent strain and despite giving repeated doses of the immunotherapeutic agent instantly claimed, no local toxicity was observed in the treated animals. Such results were surprising and could not reasonably have been predicted.

As further evidence, Dr. Cardona's declaration explains that RUTI, a vaccine obtained by the process described and claimed in the present patent application, is the first therapeutic vaccine

for the treatment of latent tuberculosis based on a non-purified mixture of antigens to reach phase I clinical trials.

In conclusion, it is respectfully submitted that the teachings of Andersen *et al.* and Chaturvedi *et al.*, taken alone or in combination, would not have led a person of ordinary skill in the art to prepare an immunotherapeutic agent by isolating cell wall fragments from a virulent *Mycobacterium tuberculosis* - complex (MTB-C) strain using a process which includes a step of homogenizing the cells in the presence of the selected non-ionic surfactant and a culturing time of at least three weeks, as instantly claimed, with a reasonable expectation of success of achieving the observed activity and effectiveness, at the time of the instant invention.

For the foregoing reasons, Applicants respectfully submit that the claims are not rendered obvious by the cited references and request that this rejection under 35 U.S.C. § 103 be reconsidered and withdrawn.

V.2 Rejection over Andersen *et al.* in view of Chaturvedi *et al.* and further in view of Unger *et al.* and Rejection over Andersen *et al.* in view of Chaturvedi *et al.* and further in view of Unger *et al.* and Parikh I.

Claims 47, 54 and 56-59 remain rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Andersen *et al.* (U.S. Patent Application Publication No.: 2002/0094336), in view of Chaturvedi *et al.*, as applied to claims 20, 28, 29, 38, and 47-55 above, and further in view of Unger *et al.* (U.S. Patent No.: 6,443,898).

Claims 47, 54 and 56-60 remain rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Andersen *et al.* (U.S. Patent Application Publication No.: 2002/0094336); in view of Chaturvedi *et al.* (Vaccine 17:2882-2887; 1999), and further in view of Unger *et al.* (U.S. Patent No.: 6,443,898) as applied to claims 28-33, 38-42, 47, 54 and 56-59 above, and Parikh I (U.S. Patent No.: 5,785,975).

Neither of the secondary references Unger *et al.* and Parikh I cures the deficiencies of the combination of Andersen *et al.* and Chaturvedi *et al.* references, since neither Unger *et al.* nor Parikh I teaches or suggests any method for preparing cell wall fragments as discussed in our previous response.

Since, as discussed above, claims 47-55 are non-obvious over Andersen *et al.* in view of Chaturvedi *et al.* then dependent claim 60, which specifies that the composition further comprises vitamin E, and dependent claims 56-59, which specify that the composition is in the form of liposomes, must also be non-obvious.

VI. Concluding Remarks

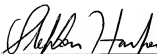
Applicants respectfully request withdrawal of the *prima facie* obviousness rejections, since it has been established, as supported by Dr. Cardona's declaration, that:

- the cited prior art references do not disclose all the claim limitations;
- it is apparent to a person having ordinary skill in the art that the method steps of the product-by-process claims currently pending and under examination, which are neither taught or suggested in the prior art, would yield a product with different structure and properties; and

- there was no reasonable expectation of success that an immunotherapeutic agent against latent tuberculosis, based on a non-purified cell extract of a virulent tuberculosis strain, that would result in effectively reducing the number of bacilli and, also importantly, without inducing toxic responses, so that it could be potentially used to significantly reduce the currently employed long-term (6 to 9 month) chemotherapy treatment, would be obtained by performing the method steps recited in the claims of the present application.

Early and favorable action in the application is respectfully. If any issues should remain, the Examiner is encouraged to contact Applicants' legal representatives at the number listed below.

Respectfully submitted,



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Attachments: Declaration of Dr. Pere-Joan Cardona, including as exhibits A) Moreira et al., *Infect. Immun.* 2002;70:2100-7; B) Turner et al., *Infect. Immun.* 2000;68:1706-9; C) Lowrie et al., *Nature* 1999 Jul 15;400(6741):269-71; D) Taylor et al., *Infect. Immun.* 2003 April; 71(4): 2192-2198; E) U.S. Pat. No. 4,724,144; F) Cochrane Database Syst. Rev 2003;(1):CD001166.

Dated: February 5, 2010

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